

PCT

RECEIVED

04 OCT 2004

WIPO

PCT



INTERNATIONAL PRELIMINARY EXAMINATION REPORT  
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 10300.204-WO	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA416)	
International application No. PCT/DK 03/00455	International filing date (day/month/year) 01.07.2003	Priority date (day/month/year) 01.07.2002
International Patent Classification (IPC) or both national classification and IPC C12N9/00		
Applicant NOVOZYMES AS et al.		

- This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 5 sheets, including this cover sheet.
  - ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 1 sheets.

- This report contains indications relating to the following items:
  - I ☒ Basis of the opinion
  - II ☐ Priority
  - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
  - IV ☐ Lack of unity of invention
  - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
  - VI ☐ Certain documents cited
  - VII ☐ Certain defects in the international application
  - VIII ☐ Certain observations on the international application

Date of submission of the demand  26.01.2004	Date of completion of this report  01.10.2004
Name and mailing address of the international preliminary examining authority:   European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer  Lejeune, R Telephone No. +31 70 340-2347 

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. **PCT/DK 03/00455**

**1. Basis of the report**

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, Pages**

1-20 as originally filed

**Claims, Numbers**

1-9 received on 30.06.2004 with letter of 30.06.2004

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the International search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.  
☐ filed together with the international application in computer readable form.  
☐ furnished subsequently to this Authority in written form.  
☐ furnished subsequently to this Authority in computer readable form.  
☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. **PCT/DK 03/00455**

---

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;  
citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes: Claims	4,5
	No: Claims	1-3,6-9
Inventive step (IS)	Yes: Claims	
	No: Claims	1-9
Industrial applicability (IA)	Yes: Claims	1-9
	No: Claims	

**2. Citations and explanations**

**see separate sheet**

**V. Reasoned statement (Continuation)**

Reference is made to the following documents:

- D1: GB-A-1 001 173 (MILES LAB) 11 August 1965 (1965-08-11)
- D2: CH 667 673 A (EIDGENOESS TECH HOCHSCHULE) 31 October 1988 (1988-10-31)
- D3: DD 153 495 A (PETROLCHEMISCHES KOMBINAT;WISS ALLUNIONSFORSCHUNGSINST F) 13 January 1982 (1982-01-13)
- D4: PATENT ABSTRACTS OF JAPAN vol. 009, no. 153 (C-288), 27 June 1985 (1985-06-27) & JP 60 030682 A (AMANO SEIYAKU KK), 16 February 1985 (1985-02-16)
- D5: US-A-5 260 202 (CLARKE PETER M ET AL) 9 November 1993 (1993-11-09)

**NOVELTY (Art. 33(2) PCT)**

Methods for fermenting a bacterium producing an enzyme of interest where one of the listed compounds is added to the medium are not disclosed in the cited prior art (D1-D5).

Nevertheless, it is general knowledge that many of the compounds listed are present in trace amounts in a many nutrient sources (glucose syrup, various hydrolysates, etc) routinely used in industrial fermentations. Therefore, the subject matter of claims 1-3 and 6-9 is not new.

The subject matter of claims 4 and 5 is new.

**INVENTIVE STEP (Art. 33(3) PCT)**

The subject matter of claims 4 and 5 is not inventive because addition of a low concentration (0.1% w/w) of any of the compounds of claim 1 or addition of 1,2-propanediol (at any concentration including infinitesimal amounts) does not have any significant effect and therefore does not solve any problem.

A positive inventive step assessment could be made (in case novelty is established)

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

---

International application No. PCT/DK 03/00455

because, although the prior art (e.g. D1-D5) discloses the use of polyethylene glycol, polypropylene glycol, sorbitol, etc during fermentations, it is not disclosed for the prevention of formation of enzyme precipitates.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/DK 03/00455

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 7 C12N9/00 C12N1/38

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, PAJ, CHEM ABS Data, WPI Data, COMPENDEX

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CH 667 673 A (EIDGENOESS TECH HOCHSCHULE) 31 October 1988 (1988-10-31) example 4	1,2,4,7, 9-13
X	DD 153 495 A (PETROLCHEMISCHES KOMBINAT;WISS ALLUNIONSFORSCHUNGSINST F) 13 January 1982 (1982-01-13) abstract; example 2	1,2,4, 9-13
X	US 5 260 202 A (CLARKE PETER M ET AL) 9 November 1993 (1993-11-09) the whole document	1-7,9-13
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

- \*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- \*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- \*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- \*&\* document member of the same patent family

Date of the actual completion of the international search

9 January 2004

Date of mailing of the international search report

21/01/2004

Name and mailing address of the ISA  
European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Lejeune, R

# INTERNATIONAL SEARCH REPORT

Inter application No  
PCT/DK 03/00455

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PATENT ABSTRACTS OF JAPAN vol. 009, no. 153 (C-288), 27 June 1985 (1985-06-27) & JP 60 030682 A (AMANO SEIYAKU KK), 16 February 1985 (1985-02-16) abstract ---	13
X	GB 1 001 173 A (MILES LAB) 11 August 1965 (1965-08-11) the whole document ---	13
A	ESTEVE-ROMERO JOSEP S ET AL: "Purification of thermamylase in multicompartment electrolyzers with isoelectric membranes: The problem of protein solubility." ELECTROPHORESIS, vol. 17, no. 7, 1996, pages 1242-1247, XP001104576 ISSN: 0173-0835 the whole document ---	1-8
A	US 4 673 647 A (BROTHERS CHARLES E ET AL) 16 June 1987 (1987-06-16) the whole document ---	1-8
A	US 4 016 039 A (SCHREIBER WOLFGANG) 5 April 1977 (1977-04-05) the whole document ---	1-13
P, X	WO 03 014339 A (KAASGAARD SVEND ; NOVOZYMES AS (DK); NOVOZYMES NORTH AMERICA INC (U) 20 February 2003 (2003-02-20) the whole document -----	1-13

# INTERNATIONAL SEARCH REPORT

Intern Application No

PCT/DR 03/00455

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
CH 667673	A	31-10-1988	CH 667673 A5	31-10-1988
DD 153495	A	13-01-1982	DD 153495 A3	13-01-1982
			SU 1306952 A1	30-04-1987
US 5260202	A	09-11-1993	AT 118249 T	15-02-1995
			AU 618413 B2	19-12-1991
			AU 4310789 A	02-04-1990
			CA 1336416 C	25-07-1995
			DE 68921063 D1	23-03-1995
			DE 68921063 T2	06-07-1995
			DK 107490 A	01-05-1990
			EP 0386222 A1	12-09-1990
			FI 96040 B	15-01-1996
			WO 9002808 A2	22-03-1990
			HU 56398 A2	28-08-1991
			IE 61268 B1	19-10-1994
			JP 2996405 B2	27-12-1999
			JP 3500969 T	07-03-1991
			ZA 8906847 A	25-07-1990
JP 60030682	A	16-02-1985	NONE	
GB 1001173	A	11-08-1965	US 3186919 A	01-06-1965
US 4673647	A	16-06-1987	DK 205886 A	07-11-1986
			EP 0201017 A2	12-11-1986
			JP 61254188 A	11-11-1986
US 4016039	A	05-04-1977	DE 2512735 A1	30-09-1976
			CH 625534 A5	30-09-1981
			DK 86876 A	23-09-1976
			IT 1058477 B	10-04-1982
			JP 51139691 A	02-12-1976
			NL 7602108 A	24-09-1976
WO 03014339	A	20-02-2003	WO 03014339 A2	20-02-2003



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property  
Organization  
International Bureau



(43) International Publication Date  
8 January 2004 (08.01.2004)

PCT

(10) International Publication Number  
WO 2004/003187 A3

(51) International Patent Classification<sup>7</sup>: C12N 9/00, 1/38

(21) International Application Number:

PCT/DK2003/000455

(22) International Filing Date: 1 July 2003 (01.07.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

PA 2002 01021

1 July 2002 (01.07.2002) DK

(71) Applicant (for all designated States except US):  
NOVOZYMES A/S [DK/DK]; Krogshøjvej 36, DK-2880  
Bagsværd (DK).

(72) Inventors; and

(75) Inventors/Applicants (for US only): KAASGAARD,  
Svend [DK/DK]; Kong Hans Allé 7, 1. idt, DK-2860  
Søborg (DK). BANKE, Niels [DK/DK]; Fremtidsvej 54,  
DK-2860 Søborg (DK). HANSEN, Kim, Uhre [DK/DK];  
Ubberup Mark 1, DK-4400 Kalundborg (DK).

(74) Common Representative: NOVOZYMES A/S; Patents,  
Krogshøjvej 36, DK-2880 Bagsværd (DK).

(81) Designated States (national): AE, AG, AL, AM, AT, AU,  
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,  
CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,  
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,  
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,  
MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC,  
SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,  
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM,  
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),  
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),  
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,  
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,  
SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the  
claims and to be republished in the event of receipt of  
amendments

(88) Date of publication of the international search report:  
18 March 2004

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: MPG ADDED TO FERMENTATION

(57) Abstract: A method for fermenting a microorganism, producing a polypeptide of interest, in a culture medium of at least 50 litres, comprising: adding one or more compounds selected from the group consisting of monopropylene glycol, ethylene glycol, trehalose, xylitol, arabitol, dulcitol, mannitol, erythritol, cellobiose and sorbitol, to the culture medium before and/or during fermentation, wherein the compound is low metabolizable.



WO 2004/003187 A3

ART 34 AMBT

EPO - DG 1

- 2.07.2004

AMENDED CLAIMS

(110)

1. A method for fermenting a bacterium, producing an enzyme of interest, in a culture  
5 medium of at least 50 litres, comprising:  
adding one or more compounds selected from the group consisting of 1,2-propandiol, 1,3-  
propandiol, ethylene glycol, trehalose, xylitol, arabitol, dulcitol, mannitol, erythritol, cellobiose,  
sorbitol and a polyether having an average molecular weight less than 1000, to the culture  
medium before and/or during fermentation, wherein the compound is low metabolizable  
10 measured by  $(OD_{III}-OD_{II})/(OD_I-OD_{II}) < 25\%$  as defined herein.
2. The method according to claim 1, wherein the bacterium is a *Bacillus* strain.
3. The method according to claim 1, wherein the enzyme is a hydrolase (class EC 3  
15 according to Enzyme Nomenclature).
4. The method according to claim 1, wherein the compound is added in an amount of least  
0.1 % (w/w) of the culture medium.
- 20 5. The method according to claim 1, wherein the compound is 1,2-propandiol.
6. The method according to claim 1, wherein in addition to the compound a salt is added to  
the fermentation medium.
- 25 7. The method according to claim 6, wherein the salt is selected from the group consisting of  
a chloride, a sulphate, a phosphate, a nitrate, and an ammonium salt.
8. The method according to claim 1, wherein the enzyme of interest is recovered.
- 30 9. The method according to claim 1, wherein the enzyme is recovered after removal of the  
bacterium.